

Article

Effects of Shade Treatments on Growth, Photosynthetic Performance, Leaf Microstructure, and Pigment Accumulation in *Horsfieldia hainanensis* Seedlings

Lin Gou ^{1,2}, Shang Shi ^{1,2}, Xi Li ^{1,2}, Hongyi Wang ^{2,3}, Ling Liu ^{1,2}, Xiao Wei ², Rong Zou ², Chenghao Zhu ^{2,*} and Jianmin Tang ^{2,*} 

¹ College of Life Sciences, Guangxi Normal University, Guilin 541006, China; forestrygoulin@163.com (L.G.); shishangfishona@163.com (S.S.); lixi020207@163.com (X.L.); liuling@mailbox.gxnu.edu.cn (L.L.)

² Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin 541006, China; 15310901899@163.com (H.W.); weixiao@gxib.cn (X.W.); zour@gxib.cn (R.Z.)

³ College of Pharmacy, Guilin Medical University, Guilin 541006, China

* Correspondence: medicalzch@163.com (C.Z.); tjm@gxib.cn (J.T.)

Abstract

In this study, three-year-old seed-derived seedlings of *Horsfieldia hainanensis* Merr. were subjected to 0%, 25%, 50%, and 75% shade treatments in a nursery shade-net experiment from October 2025 to June 2026. Each treatment included three replicates, with three seedlings per replicate. The results showed that the light environment significantly affected growth. Seedlings under 0% shade showed severe wilting and mortality; therefore, this treatment was treated as a mortality outcome and excluded from subsequent statistical comparisons, which were conducted among the 25%, 50%, and 75% shade treatments. Within this range, plant height, ground diameter, crown width, leaf traits, and above- and belowground biomass increased with shade level, with the highest values observed under 75% shade. The 75% shade treatment was associated with higher maximum net photosynthetic rate (P_{max}), apparent quantum yield (AQY), initial carboxylation efficiency (α), and CO_2 -saturated net photosynthetic rate (A_{max}), together with lower light compensation point (LCP) and carbon dioxide compensation point (CDCP), indicating improved light- and CO_2 -response performance within the tested shade range. Leaf thickness, epidermal thickness, and palisade and spongy parenchyma thicknesses increased with shade level, whereas variation in stomatal traits was mainly reflected in stomatal density. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids increased under stronger shading, showing clear variation in pigment accumulation along the shade gradient. Growth, biomass, photosynthetic capacity, leaf anatomical traits, and pigment contents were positively correlated, whereas the light saturation point (LSP), LCP, carbon dioxide saturation point (CDSP), and CDCP were generally negatively associated with these traits. Among the surviving treatments tested, the 75% shade treatment produced the best seedling performance and may be suitable for nursery cultivation, although further validation at different sites and in different seasons is needed. These findings provide practical guidance for artificial propagation, seedling production, plantation establishment, and light-environment management of this species.

Keywords: chlorophyll accumulation; *Horsfieldia hainanensis*; light acclimation; nursery cultivation; photosynthetic response; seedling survival; shade-net treatment



Academic Editor: Junfei Gu

Received: 29 May 2026

Revised: 14 July 2026

Accepted: 26 July 2026

Published: 2 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/)

[Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Horsfieldia hainanensis Merr. is an evergreen tree belonging to the family Myristicaceae and the genus *Horsfieldia* and is also known by several local names, including Hainan Hesifeimu, shuipipa, and jiaiyuguo [1,2]. The species is mainly distributed in Guangxi, Hainan, and Yunnan and is one of the indicator species of tropical rainforests [3–5]. It usually occurs in moist, shaded, dense forests in valleys and hills at elevations of 400–450 m and prefers warm, humid, and well-drained habitats [6]. Because of its restricted geographic distribution, human disturbance, and illegal logging, the habitat of *H. hainanensis* has become severely fragmented and its population size has continued to decline. It is currently endangered and has been listed in China as a plant species with extremely small populations and as a second-class national key protected wild plant [7].

Previous studies on *H. hainanensis* have provided important information on cultivation and propagation [8–10], heavy-metal stress responses [11], chemical constituents [12–14], community composition [15–17], genetic diversity [18–21], and physiological traits [22–25]. These studies have improved our understanding of the conservation value, habitat characteristics, and physiological sensitivity of this endangered species. In particular, existing evidence suggests that successful seedling cultivation and establishment of *H. hainanensis* are closely related to light availability because inappropriate irradiance may restrict photosynthesis, leaf development, pigment accumulation, and biomass production. However, most previous studies have focused on single aspects of seedling performance or general physiological responses, whereas integrated evaluations of growth allocation, photosynthetic response curves, leaf anatomical traits, stomatal characteristics, pigment regulation, and trait plasticity under controlled shade gradients remain limited. Therefore, the optimal shade level for nursery cultivation and early seedling management of *H. hainanensis* is still not sufficiently clear. Inappropriate irradiance may inhibit photosynthesis, leaf development, and biomass accumulation, thereby affecting seedling growth and establishment. Therefore, identifying an appropriate shading level is crucial for enhancing seedling growth performance and providing support for artificial propagation and cultivation management. Light is a key ecological factor regulating plant growth, development, and photosynthate accumulation [26,27]. Different light environments may alter leaf thickness, the differentiation of palisade and spongy tissues, stomatal traits, and other anatomical characteristics [28] and may also regulate chlorophyll contents and their relative proportions, thereby affecting light capture, light-use efficiency, and biomass accumulation [29]. Light is essential for photosynthesis [30] and plays a central role in growth, development, and biomass production throughout the plant life cycle [31]. Under different light intensities and light qualities, plants often adjust their morphology, physiological activity, and metabolism to improve light-use efficiency [32]. When light conditions deviate from the suitable range, however, plants may show reduced photosynthetic efficiency, inhibited growth, and disturbed pigment metabolism [33]. Under low light, the activity of related enzymes may be constrained, photosynthesis may decline, and carbohydrate synthesis and accumulation in leaves may decrease, thereby limiting individual growth and reproduction [34]. Excessive light may induce photoinhibition or even damage photosystems [35]. Therefore, seedlings can grow normally only under appropriate light conditions.

Natural regeneration of *H. hainanensis* is limited, and seedling survival in natural habitats is low. This limitation may be closely associated with the species' narrow habitat range and its dependence on moist, shaded understory environments in valleys and hills. During early seedling establishment, light conditions may act as a key ecological filter: excessive irradiance can increase photoinhibition risk and water stress, whereas excessive shade may limit carbon assimilation and biomass accumulation [36]. Therefore, clarifying how seedlings respond to different shade levels is essential for understanding the physio-

logical basis of poor natural recruitment and for developing nursery light-management strategies that better match the species' understory habitat requirements. Although previous studies have examined the physiology and light responses of *H. hainanensis*, few have simultaneously evaluated growth allocation, photosynthetic response curves, leaf anatomical plasticity, stomatal traits, and pigment regulation under controlled shade gradients. Therefore, the novelty of this study lies in linking structural and physiological acclimation mechanisms to practical nursery management. In this study, *H. hainanensis* seedlings were subjected to different shade treatments, and growth traits, photosynthetic parameters, leaf microstructure, and photosynthetic pigment contents were systematically measured. The objective was to evaluate how shade-net treatments affect seedling growth, photosynthetic performance, leaf anatomical traits, and pigment accumulation and to identify the shade treatment that was most favorable for seedling performance under the tested nursery conditions.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted in the nursery of the Guangxi Institute of Botany, Chinese Academy of Sciences, Yanshan District, Guilin, China (25°01' N, 110°17' E; approximately 180 m a.s.l.). The region has a mid-subtropical monsoon climate, with abundant sunshine and rainfall, mild temperatures, and an annual mean temperature of approximately 19.2 °C [37]. The shade experiment was conducted from October 2025 to June 2026. According to long-term monthly climate averages for Guilin, the corresponding experimental months had a mean air temperature of approximately 16.7 °C, cumulative precipitation of approximately 1295 mm, and a mean relative humidity of approximately 75.0%.

A fully open area without natural shade was selected. Black polyethylene shade nets were used to establish the shade treatments. Shade levels were defined according to the nominal shading rates provided by the shade-net manufacturer and included 75%, 50%, and 25% shade. The 0% shade treatment was not covered by a shade net and served as the full-light treatment. All shade structures were built with supports of the same specifications. Shade nets covered the top and sides of each structure, while ventilation was kept consistent. The shade-net roof was installed approximately 2 m above the ground, and the side nets extended to approximately 2 m above the ground while allowing natural ventilation. The distance between the shade net and the seedling canopy was maintained to avoid direct contact with the plants. During the experiment, water, nutrients, and daily management were kept consistent among treatments, and light availability was the only intended treatment difference.

It should be noted that the shading levels in this study represent shade-net treatments rather than continuously measured irradiance gradients. PPFD, daily light integral, seasonal variation in light availability, and microclimatic variables such as air temperature, relative humidity, vapor pressure deficit, wind exposure, and substrate moisture were not continuously monitored under each shade structure. Therefore, the observed seedling responses should be interpreted as responses to integrated shade-net treatment conditions rather than to light intensity alone.

2.2. Plant Materials

The plant material consisted of three-year-old *H. hainanensis* seedlings collected from the germplasm nursery of the Guangxi Institute of Botany, Chinese Academy of Sciences. The material was identified as *H. hainanensis* by Researcher Xiao Wei of the Guangxi Institute of Botany based on morphological characteristics. Four nominal shade treatments were established: 75%, 50%, 25%, and 0% shade. One physical experimental structure or

plot was used for each treatment, and seedlings within the same experimental structure or plot were used as seedling-level observational units. Each treatment initially included nine seedlings, resulting in a total of 36 seedlings. Because seedlings within the same experimental structure or plot shared the same physical environment, these seedling-level observations should not be regarded as fully independent treatment replicates. All seedlings were managed under the same water and nutrient conditions. Before treatment initiation, seedlings with similar growth status were selected to minimize initial differences in plant size. The initial seedling heights were 36.27 ± 3.07 , 34.97 ± 4.41 , 35.07 ± 6.59 , and 33.90 ± 6.88 cm under the 0%, 25%, 50%, and 75% shade treatments, respectively. No significant difference in initial seedling height was detected among treatments before shade treatment initiation ($p = 0.961$). Seedlings under the 0% shade treatment began to show severe wilting two months after treatment initiation, and all seedlings had died by two and a half months after treatment initiation. At the end of the experiment, no seedlings survived under the 0% shade treatment, corresponding to a survival rate of 0% and a mortality rate of 100%. Because complete data on growth performance, photosynthetic characteristics, leaf anatomical structure, and pigment contents could not be obtained after seedling mortality, the 0% shade treatment was recorded only as a mortality outcome.

2.3. Measurements

2.3.1. Growth Trait Measurements

At the end of the experiment, three surviving seedlings were randomly selected from each of the 25%, 50%, and 75% shade treatments for growth measurements. No seedlings from the 0% shade treatment were measured because all seedlings had died before the final growth assessment. To reduce selection bias, seedlings were selected only from living plants that showed no visible mechanical damage or symptoms of disease or pest infestation. These seedlings were treated as seedling-level observational units rather than fully independent treatment replicates because seedlings within the same treatment structure or plot shared the same physical environment. Seedling height, ground diameter, crown width, leaf number, root length, and root diameter were measured for each seedling. Ground diameter (diameter at the soil mark at the base of the stem) and main root diameter (root-base diameter) were measured using a vernier caliper. Seedling height (from the basal diameter position to the base of the terminal bud), crown width (mean diameter in the east–west and north–south directions), and main root length (from the root–stem junction to the tip of the longest root) were measured using a tape measure. The number of fully expanded, healthy leaves was counted manually. Aboveground and belowground fresh weights were measured using an electronic balance. Samples were heated at 105 °C for 30 min and then dried at 65 °C to constant mass to determine aboveground and belowground dry biomass. Measurements were performed on sunny days, and the data were recorded immediately. All measurements were completed within 24 h. Each trait was measured three times, and the mean value was used [38].

2.3.2. Light-Response Curve Measurements

Light-response curves were measured on clear mornings from 09:00 to 12:00 in mid-April 2026 using an LI-6800 portable photosynthesis system (LI-COR, Lincoln, NE, USA) equipped with a 6800-01 fluorescence chamber. Intact, healthy leaves from vigorous plants were selected under each light environment. Three leaves were selected from each plant, and each measurement was repeated three times. Before measurement, leaves were pre-illuminated for 15–30 min at a photosynthetic photon flux density (PPFD) of 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to ensure full stomatal opening. During measurement, the reference CO_2 concentration (CO_{2_s}) was maintained at 400 $\mu\text{mol mol}^{-1}$, the flow rate at 500 $\mu\text{mol s}^{-1}$,

the pressure difference (ΔP) at 0.1 kPa, the relative humidity (RH_{air}) at 60%, and the leaf-chamber temperature at 25 °C. H₂O control was enabled, and the mixing fan speed was set to 10,000 rpm. The automatic “Light_Response” program was used. Incident PPFD (Q_{in}) was set in descending order at 1800, 1500, 1200, 900, 600, 300, 200, 150, 100, 70, 30, and 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, with each step lasting 120–180 s. The Pn–PPFD curve was fitted using Leaf Photosynthesis Model software version 4.1.1 and a modified rectangular hyperbola model, with PPFD on the x -axis and net photosynthetic rate (Pn) on the y -axis [39].

In the model, Pn is the net photosynthetic rate; AQY is the initial slope of the light-response curve; β and γ are fitting coefficients; PPFD is the photosynthetic photon flux density; and R_d is the dark respiration rate. After model validation, the fitted curves showed good agreement with the observed data. The light saturation point (LSP), maximum net photosynthetic rate (P_{max}), and light compensation point (LCP) were calculated from the fitted model.

$$P_n = AQY \frac{1 - \beta PPFD}{1 - \gamma PPFD} PPFD - R_d \quad (1)$$

$$LSP = \frac{\sqrt{\frac{\beta + \gamma}{\beta}} - 1}{\gamma} \quad (2)$$

$$P_{max} = AQY \left(\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma} \right) - R_d \quad (3)$$

$$LCP = \frac{AQY - \gamma R_d - \sqrt{(\gamma R_d - AQY)^2 - 4\beta * AQY * R_d}}{2\alpha\beta} \quad (4)$$

2.3.3. CO₂-Response Curve Measurements

CO₂-response curves were measured on clear mornings from 09:00 to 12:00 in mid-April 2026 using the same Li-6800 portable photosynthesis system and fluorescence chamber (LI-COR, Lincoln, NE, USA). Intact, healthy leaves from vigorous plants were selected under each light environment. Three leaves were selected from each plant, and each measurement was repeated three times. Before measurement, leaves were pre-illuminated for 15–30 min at a PFD of 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to ensure full stomatal opening. During measurement, CO₂_s was maintained at 400 $\mu\text{mol mol}^{-1}$, flow rate at 500 $\mu\text{mol s}^{-1}$, ΔP at 0.1 kPa, RH_{air} at 60%, leaf-chamber temperature at 25 °C, and fan speed at 10,000 rpm with H₂O control enabled.

The automatic “CO₂_Response” program was used to measure the photosynthetic CO₂-response curves. Starting from the ambient CO₂ concentration, CO₂ was first decreased, then returned to ambient concentration, and finally increased to the maximum level. The reference CO₂ concentration was set to 400, 300, 200, 100, 50, 400, 600, 800, 1000, 1200, 1500, 1800, and 2000 $\mu\text{mol mol}^{-1}$, with each step lasting 120–180 s. The Pn–Ci curve was fitted using Leaf Photosynthesis Model software 4.1.1 and a modified rectangular hyperbola model, with the intercellular CO₂ concentration (Ci) on the x -axis and Pn on the y -axis.

In the model, Pn is the net photosynthetic rate; Ci is the intercellular CO₂ concentration; α is the initial carboxylation efficiency of the CO₂-response curve; γ and β are fitting coefficients; and R_p is the photorespiration rate. The CO₂ compensation point (CDCP) was obtained when Pn equaled zero. The CO₂ saturation point (CDSP) and CO₂-saturated net photosynthetic rate (A_{max}) were calculated from the fitted model.

$$P_n = \alpha \frac{1 - \beta C_i}{1 + \gamma C_i} C_i - R_p \quad (5)$$

$$CDCP = \frac{\alpha - \gamma R_p - \sqrt{(\alpha - \gamma R_p)^2 - 4\alpha\beta R_p}}{2\alpha\beta} Ci - R_p \quad (6)$$

$$CDSP = \frac{\sqrt{\frac{\beta + \gamma}{\beta}} - 1}{\gamma} \quad (7)$$

$$A_{\max} = \alpha \left(\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma} \right)^2 - R_p \quad (8)$$

2.3.4. Leaf Microstructure Measurements

Leaf microstructure was observed according to the method of Pan et al. (2023) [40]. Three mature, healthy leaves from the middle part of each plant were collected and washed with deionized water to remove surface dust. Leaf pieces of approximately 5 mm × 5 mm were cut from the middle part of the leaf while avoiding the main vein and leaf margin, immediately fixed in 2.5% glutaraldehyde solution (pH 7.2), and then stored at 4 °C. Samples were then dehydrated through a graded ethanol series (30%, 50%, 70%, 85%, and 90% ethanol once each and 100% ethanol twice; 15 min per step), dried at the critical point, mounted on specimen stubs, and sputter-coated with gold. The upper epidermis, lower epidermis, and stomatal apparatus were observed using a scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany), and ten fields of view were randomly observed for each sample. Axio Vision SE64 Rel.4.8 software was used to measure stomatal length (SL), stomatal width (SW), stomatal area (SA), and stomatal density (SD). Stomatal density was calculated as the number of stomata per field of view divided by the field area, and stomatal area was calculated as $SA = \pi \times SL \times SW/4$, where $\pi = 3.14$.

Leaf paraffin sections were prepared according to Li (1978) [41]. Three healthy leaves with similar orientation and growth status were collected from each plant. Leaf segments of approximately 10 mm × 10 mm were cut transversely while avoiding the main vein and leaf margin and then fixed in FAA solution (70% ethanol/formalin/acetic acid = 90:5:5). Samples were placed in a dehydration box and subjected to graded ethanol and xylene dehydration and wax infiltration. After infiltration, tissues were embedded in paraffin, sectioned, dewaxed, and rehydrated. Sections were observed and photographed under an optical microscope, and CaseViewer 2.4 image analysis software was used to measure microstructural parameters. The measured traits included upper epidermal thickness (UET), lower epidermal thickness (LET), leaf thickness (LT), palisade parenchyma thickness (PT), spongy parenchyma thickness (ST), and the ratio of palisade to spongy parenchyma thickness (PT/ST). Ten fields of view were randomly selected for each trait measurement.

2.3.5. Chlorophyll Content Measurements

Three leaves with similar maturity, position, and size were collected from each plant for chlorophyll measurements. Intact leaf tissue (0.2 g) was accurately weighed and placed in a 25 mL volumetric flask. Samples were soaked in 95% ethanol in darkness for 24 h. Absorbance was measured at 470, 649, and 665 nm using an Alpha 1502 UV-visible spectrophotometer (Shanghai Puyuan Instrument Co., Ltd., Shanghai, China). Chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll [Chl(a+b)], carotenoids (Car), and the ratio of chlorophyll a to chlorophyll b (Chl a/Chl b) were calculated according to Li (2000) [42].

$$C_a = 13.95A_{665} - 6.88A_{649} \quad (9)$$

$$C_b = 24.96A_{649} - 7.32A_{665} \quad (10)$$

$$Car = \frac{1000A_{470} - 2.05C_a - 114.8C_b}{245} \quad (11)$$

2.3.6. Data Processing

The 0% shade treatment was treated as a formal survival outcome because all seedlings under this treatment died before the final physiological and anatomical measurements. Therefore, the 0% shade treatment was not included in the ANOVA of continuous functional traits, and survival under full-light exposure was reported descriptively. For the 25%, 50%, and 75% shade treatments, seedling-, leaf-, and field-of-view-level measurements were used as observational or subsampling data. Because each shade treatment was represented by one physical experimental structure or plot, seedlings within the same treatment shared the same physical environment and were not regarded as fully independent treatment replicates. Thus, one-way analysis of variance (ANOVA) was used only as an exploratory comparison among shade-associated observational data. Duncan's multiple range test was used for post hoc comparisons at a significance level of $p < 0.05$. The statistical results were interpreted as treatment-associated differences under the present experimental conditions rather than as strict inferences based on independently replicated shade structures.

Origin 2021 (Origin Lab Corporation, Northampton, MA, USA) and RStudio 4.5.3 (RStudio, Boston, MA, USA) were used to visualize functional traits. Pearson correlation analysis was used to evaluate relationships among functional traits. Because the correlation analysis was based on observational trait data, the results were interpreted as associations among traits rather than evidence of causal relationships. The phenotypic plasticity index (PPI) was calculated as $PPI = (V_{max} - V_{min}) / V_{max}$.

3. Results

3.1. Growth Traits

As shown in Figures 1 and 2, shade treatment markedly affected the survival and morphological growth of *H. hainanensis* seedlings. Seedlings under 0% shade began to show severe wilting two months after treatment initiation, and all seedlings had died by two and a half months after treatment initiation, corresponding to a survival rate of 0% and a mortality rate of 100%. Therefore, the 0% shade treatment was treated as a mortality outcome and was not included in the statistical comparisons of growth traits.

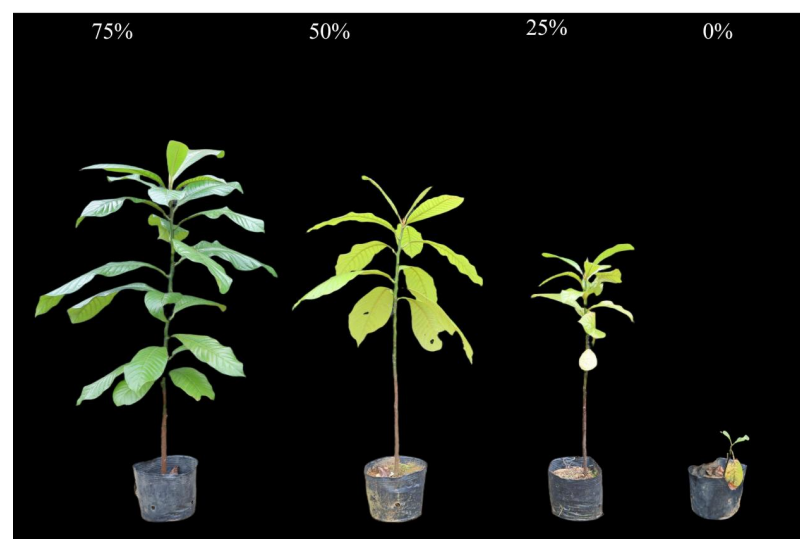


Figure 1. Morphological characteristics of *Horsfieldia hainanensis* seedlings under different shading treatments. Images show representative seedlings from the 0%, 25%, 50%, and 75% shade treatments. Each treatment initially included nine seedlings. Seedlings under 0% shade died before the final growth measurements, whereas seedling traits under 25%, 50%, and 75% shade were evaluated using surviving seedlings.

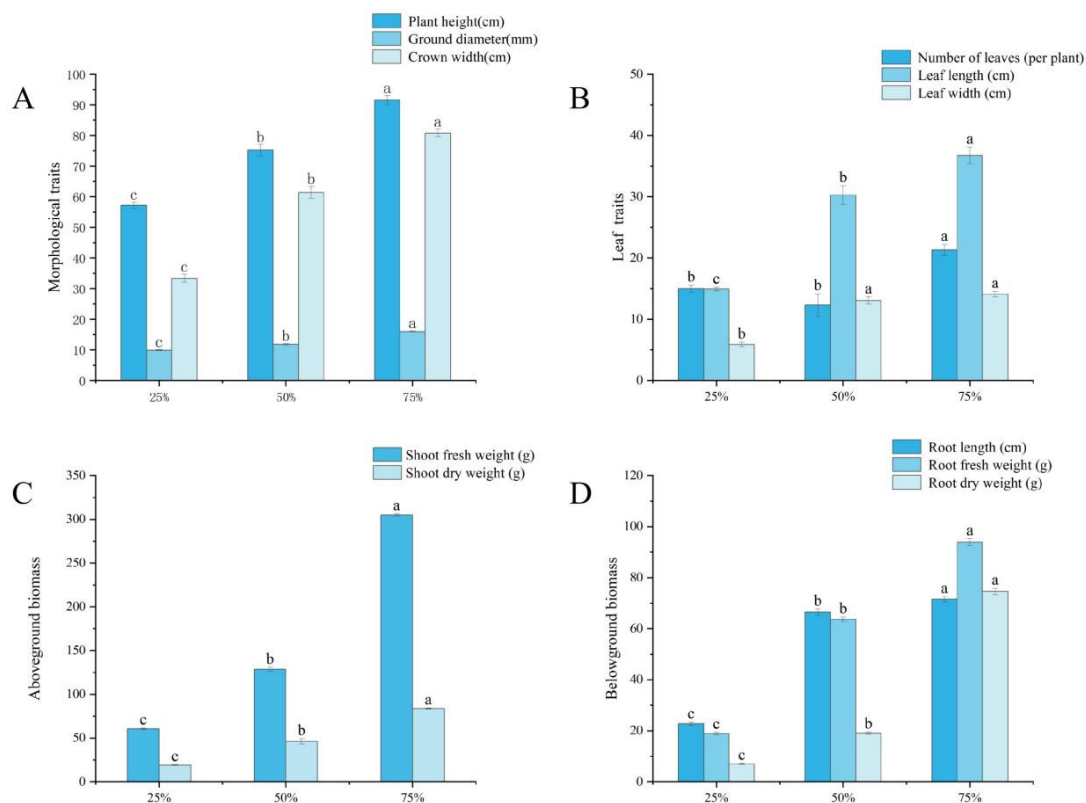


Figure 2. Growth traits and biomass of *Horsfieldia hainanensis* seedlings under different shading treatments. (A) Morphological growth traits; (B) leaf traits; (C) aboveground fresh and dry weights; (D) belowground traits. Values are means $\pm$ SE based on surviving seedlings under the 25%, 50%, and 75% shade treatments. For each surviving shade treatment, $n = 3$ seedlings. Different lowercase letters indicate significant differences among shade treatments at $p < 0.05$.

Within the tested surviving shade range of 25–75%, morphological traits differed among shade treatments (Figure 2A). Leaf traits showed a trend similar to that of the morphological traits (Figure 2B). With increasing shade, leaf number, leaf length, and leaf width generally increased; the 75% shade treatment produced the highest values overall, whereas the 25% shade treatment produced the lowest values. Aboveground fresh and dry weights differed markedly among shade treatments (Figure 2C), and their trends were consistent with those of seedling height and crown width, with the highest values under 75% shade, intermediate values under 50% shade, and the lowest values under 25% shade. Belowground traits also responded significantly (Figure 2D). Root length and belowground fresh and dry weights increased with increasing shade within the tested 25–75% range; among these treatments, root development was greatest under the 75% shade treatment and lowest under the 25% shade treatment. These results indicate that the growth and biomass accumulation of *H. hainanensis* seedlings differed among the tested shade treatments. Within the surviving shade range of 25–75%, the 75% shade treatment produced the highest values for seedling height, crown expansion, leaf development, and above- and belowground biomass accumulation, followed by the 50% shade treatment, whereas the 25% shade treatment showed the lowest values.

3.2. Photosynthetic Characteristics

3.2.1. Light-Response Curves

As shown in Figure 3, the net photosynthetic rate (P_n) of *H. hainanensis* seedling leaves increased rapidly with increasing PFD and gradually approached a plateau under high irradiance. Compared with the 25% shade treatment, AQY under 75% shade increased

by 94.6%, and P_{max} increased by 163.5%, from 1.5918 to 4.1942 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. In contrast, LSP decreased by 34.6%, and LCP decreased by 65.4%. Across the tested surviving shade treatments, P_n followed the order 75% shade > 50% shade > 25% shade, indicating that photosynthetic capacity increased with shade level within the tested 25–75% range. In particular, under moderate to high light intensities, the 75% shade treatment consistently maintained the highest P_n , followed by the 50% shade, while the 25% shade treatment showed the lowest values.

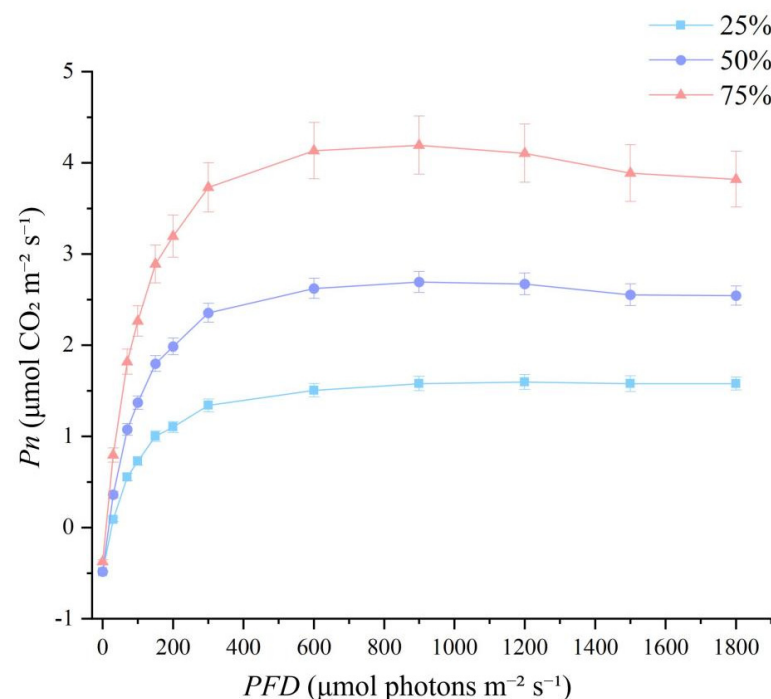


Figure 3. Light-response curves of *Horsfieldia hainanensis* seedlings under different shading treatments.

Table 1 further shows that light-response parameters differed significantly among shade levels. With increasing shade, the apparent quantum yield (AQY) increased from 0.0258 to 0.0502, and P_{max} increased from 1.5918 to 4.1942 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. P_{max} under the 75% shade was significantly higher than that under the 50% and 25% shade, whereas the 25% shade produced the lowest value. LSP decreased from 1234.5608 to 807.2767 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and LCP decreased from 23.9174 to 8.2650 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. R_d did not differ significantly between the 25% and 50% shade treatments but was significantly lower under the 75% shade. Thus, within the tested shade range, the 75% shade treatment was associated with higher AQY and P_{max} and lower LCP and R_d , suggesting better light-response performance under low irradiance. The fitted light-response curves showed good agreement with the observed values, with R^2 values ranging from 0.9991 to 0.9995 among shade treatments, indicating high model-fitting quality.

Table 1. Light-response parameters of *Horsfieldia hainanensis* seedlings under different shading treatments.

Shading Level	AQY ($\text{mol CO}_2 \text{ mol}^{-1} \text{ photons}$)	P_{max} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	LSP ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	LCP ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	R_d ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	R^2
25%	0.0258 ± 0.0010 c	1.5918 ± 0.0786 c	1234.5608 ± 16.3613 a	23.9174 ± 1.1144 a	0.4878 ± 0.0020 a	0.9991 ± 0.00020
50%	0.0369 ± 0.0015 b	2.6883 ± 0.1153 b	904.2741 ± 10.5587 b	15.2422 ± 0.8633 b	0.4689 ± 0.0045 a	0.9993 ± 0.0001
75%	0.0502 ± 0.0023 a	4.1942 ± 0.3177 a	807.2767 ± 11.7043 c	8.2650 ± 0.8770 c	0.3830 ± 0.0195 b	0.9995 ± 0.0000

Note: Values are means $\pm$ SE. Different lowercase letters within the same row indicate significant differences among shade treatments at $p < 0.05$. P_{max} , maximum net photosynthetic rate; AQY, apparent quantum yield; LSP, light saturation point; LCP, light compensation point; R_d , dark respiration rate; R^2 , coefficient of determination of the fitted light-response curve. Units: P_{max} and R_d , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; LSP and LCP, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; AQY is expressed as $\text{mol CO}_2 \text{ mol}^{-1} \text{ photons}$, whereas R^2 is dimensionless.

3.2.2. CO₂-Response Curves

As shown in Figure 4, P_n generally increased rapidly with increasing intercellular CO₂ concentration (C_i) and then gradually stabilized. P_n differed markedly among shade treatments. In the moderate- to high-CO₂ concentration range, the overall order was 75% shade > 50% shade > 25% shade.

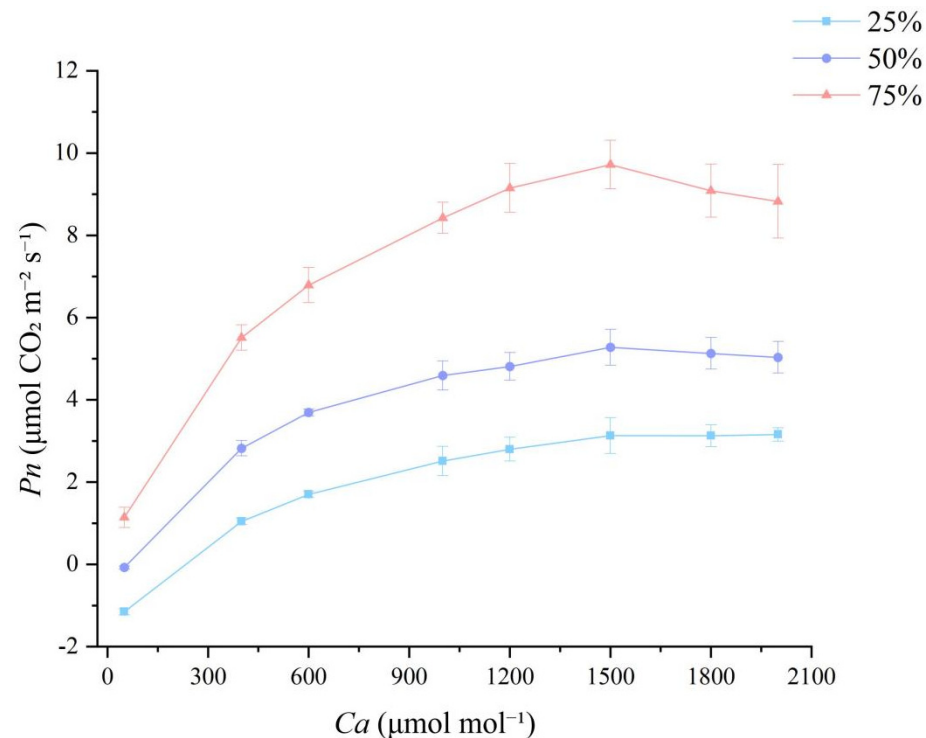


Figure 4. CO₂-response curves of *Horsfieldia hainanensis* seedlings under different shading treatments.

As shown in Table 2, shade level significantly affected the CO₂-response parameters in *H. hainanensis* seedlings. Compared with the 25% shade treatment, A_{max} under the 75% shade increased by 196.5%, while CDSP and CDCP decreased by 26.0% and 73.2%, respectively. A_{max} was highest under 75% shade ($9.5294 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), significantly higher than that under 50% and 25% shade. The 50% shade treatment ranked second ($5.1323 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), whereas the 25% shade treatment had the lowest value ($3.2137 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). The trend in the initial carboxylation efficiency (α) was consistent with that of A_{max} , with significantly higher values under 75% and 50% shade than under 25% shade. CDSP followed the order 25% shade > 50% shade > 75% shade, and the value under 75% shade was significantly lower than that under 25% shade. CDCP also decreased significantly with increasing shade and reached the lowest value under 75% shade. R_p did not differ significantly among treatments, although it was highest under 25% shade and lowest under 75% shade. Therefore, within the tested shade range, the 75% shade treatment showed higher α and A_{max} and lower CDSP and CDCP, indicating better CO₂-response performance based on the fitted P_n - C_i curve. The fitted CO₂-response curves also showed good agreement with the observed values, with R^2 values ranging from 0.9879 to 0.9942 among shade treatments, indicating acceptable model-fitting quality.

Table 2. CO₂-response parameters of *Horsfieldia hainanensis* seedlings under different shading treatments.

Shading Level	α [($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)/($\mu\text{mol mol}^{-1}$)]	Amax ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	CDSP ($\mu\text{mol mol}^{-1}$)	CDCP ($\mu\text{mol mol}^{-1}$)	Rp ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	R ²
25%	0.0218 ± 0.0010 b	3.2137 ± 0.1941 c	1335.6860 ± 107.9738 a	147.2733 ± 7.6623 a	2.3170 ± 0.1649 a	0.9942 ± 0.0010
50%	0.0349 ± 0.0038 a	5.1323 ± 0.3945 b	1113.3213 ± 21.2852 ab	69.1134 ± 0.8888 b	1.9446 ± 0.1488 a	0.9909 ± 0.0029
75%	0.0441 ± 0.0044 a	9.5294 ± 0.5922 a	987.8442 ± 58.5765 b	39.4591 ± 4.7713 c	1.6166 ± 0.3178 a	0.9879 ± 0.0039

Note: Values are means ± SE. Different lowercase letters within the same row indicate significant differences among shade treatments at $p < 0.05$. Amax, CO₂-saturated net photosynthetic rate; α , initial carboxylation efficiency; CDSP, CO₂ saturation point; CDCP, CO₂ compensation point; Rp, photorespiration rate; R², coefficient of determination of the fitted CO₂-response curve. Units: Amax and Rp, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; α , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ per $\mu\text{mol mol}^{-1}$; CDSP and CDCP, $\mu\text{mol mol}^{-1}$; R² is dimensionless.

3.3. Leaf Microstructure

3.3.1. Leaf Anatomical Structure

As shown in Figure 5, the leaf anatomical structure of *H. hainanensis* seedlings differed markedly among shade treatments. With increasing shade, leaves gradually became thicker, and both palisade and spongy tissues tended to thicken. Leaf and mesophyll tissues showed greater measured thickness under 75% shade, whereas leaves under 25% shade had lower thickness values and relatively weaker tissue differentiation. Figure 6 shows that shade treatment was associated with clear differences in leaf anatomical parameters. LT increased from 254.167 μm under 25% shade to 286.667 μm under 50% shade and 323.667 μm under 75% shade, corresponding to an approximately 27.4% increase from 25% to 75% shade. UET increased from 20.933 μm under 25% shade to 29.067 μm under 75% shade, representing an approximately 38.9% increase. PT increased from 135.500 to 158.700 μm , and ST increased from 90.167 to 114.033 μm from 25% to 75% shade, corresponding to increases of approximately 17.1% and 26.5%, respectively. LET was highest under 50% shade, whereas the 25% and 75% shade treatments showed lower values. The PT/ST ratio did not differ significantly among treatments. These results indicate that the observed increase in leaf thickness under stronger shade was mainly associated with increases in epidermal and mesophyll tissue thickness, whereas the relative allocation between palisade and spongy tissues remained relatively stable.

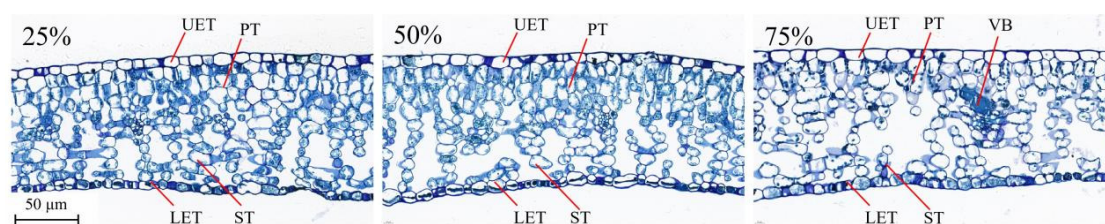


Figure 5. Leaf anatomical structure of *Horsfieldia hainanensis* seedlings under different shading treatments. Images show representative paraffin sections of leaves from the 25%, 50%, and 75% shade treatments observed under light microscopy. Scale bars and magnification are shown in each panel. UET, upper epidermal thickness; PT, palisade parenchyma thickness; ST, spongy parenchyma thickness; LET, lower epidermal thickness; VB, vascular bundle. Arrows or labels indicate the major anatomical tissues.

3.3.2. Leaf Epidermal Traits

As shown in Figures 7 and 8, the leaf epidermal traits of *H. hainanensis* seedlings showed no obvious overall differences among shade treatments. The upper epidermis was relatively smooth, and no stomata were observed on its surface. Stomata were mainly distributed on the lower epidermis, and the stomatal apparatus was generally complete and elliptical. The ornamentation of the upper and lower epidermis and stomatal morphology changed little among shade treatments.

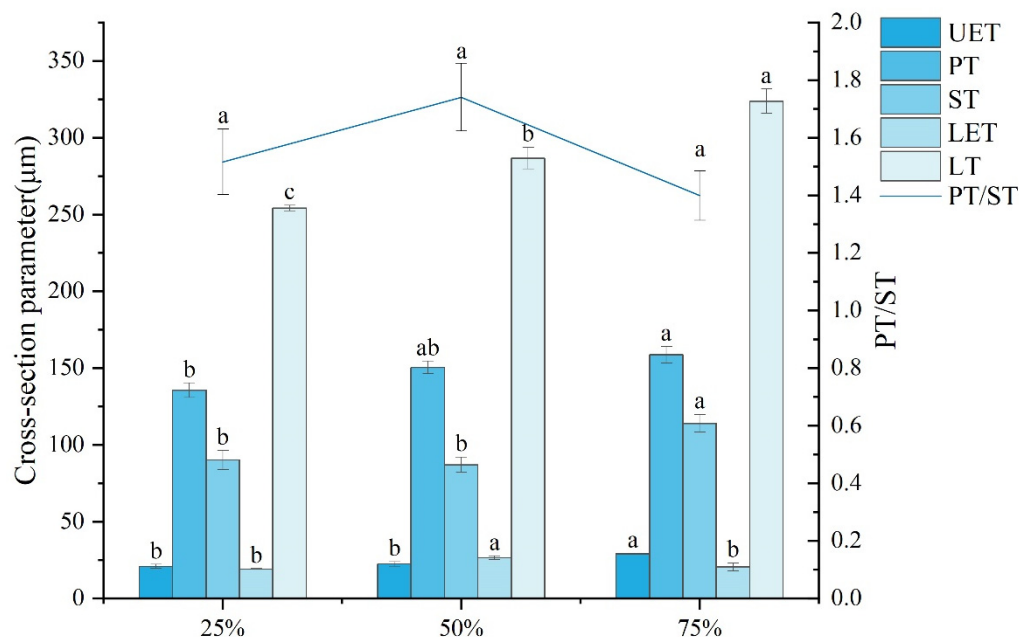


Figure 6. Leaf anatomical parameters of *Horsfieldia hainanensis* seedlings under different shading treatments. Note: LT, leaf thickness; UET, upper epidermal thickness; PT, palisade parenchyma thickness; ST, spongy parenchyma thickness; LET, lower epidermal thickness; PT/ST, ratio of palisade parenchyma thickness to spongy parenchyma thickness. Values are means $\pm$ SE. Different lowercase letters indicate significant differences among shade treatments at $p < 0.05$. Measurements were obtained from leaf cross-sectional microscopic images, and scale bars are shown in Figure 5.

According to Figure 8, stomatal length (SL, μm), stomatal width (SW, μm), and stomatal area (SA, μm^2) did not differ significantly among shade treatments. In contrast, stomatal density (SD), calculated as the number of stomata per unit epidermal area and expressed as stomata mm^{-2} , differed significantly among treatments. SD under 25% shade was significantly lower than that under 50% and 75% shade, whereas no significant difference was detected between 50% and 75% shade. These results indicate that, within the tested shade range, variation in the leaf epidermal traits of *H. hainanensis* seedlings was mainly reflected in stomatal density per unit leaf area rather than in individual stomatal size. Stomatal morphology remained relatively stable among shade treatments.

3.4. Chlorophyll Content

As shown in Figure 9, photosynthetic pigment contents in *H. hainanensis* seedling leaves differed markedly among shade treatments. With increasing shade, Chl a, Chl b, Chl(a+b), and Car contents generally increased. Overall, pigment contents were highest under 75% shade and lowest under 25% shade. Chl a, Chl b, and Chl(a+b) were significantly higher under 75% shade. Different pigment components responded differently to shade. Compared with Chl a, Chl b increased more clearly under 75% shade, causing the Chl a/Chl b ratio to be higher under 50% shade but lower under 75% shade. This pattern suggests that leaves increased their relative Chl b content under high shade to improve low-light absorption. Car content also increased significantly under 75% shade.

3.5. Plasticity Index

As shown in Figure 10, the plasticity indices differed among functional trait categories. Pigment-related traits and major photosynthetic parameters showed relatively high plasticity, whereas stomatal size traits showed relatively low plasticity. Among the photosynthetic parameters, Amax and Pmax had relatively high plasticity indices of approximately 0.66 and 0.62, respectively. Among the leaf anatomical traits, the plasticity indices of UET, LET,

ST, and LT were approximately 0.28, 0.28, 0.23, and 0.21, respectively, whereas PT showed a relatively low value of approximately 0.15. The plasticity of leaf anatomical traits followed the order UET and LET > ST > LT > PT.

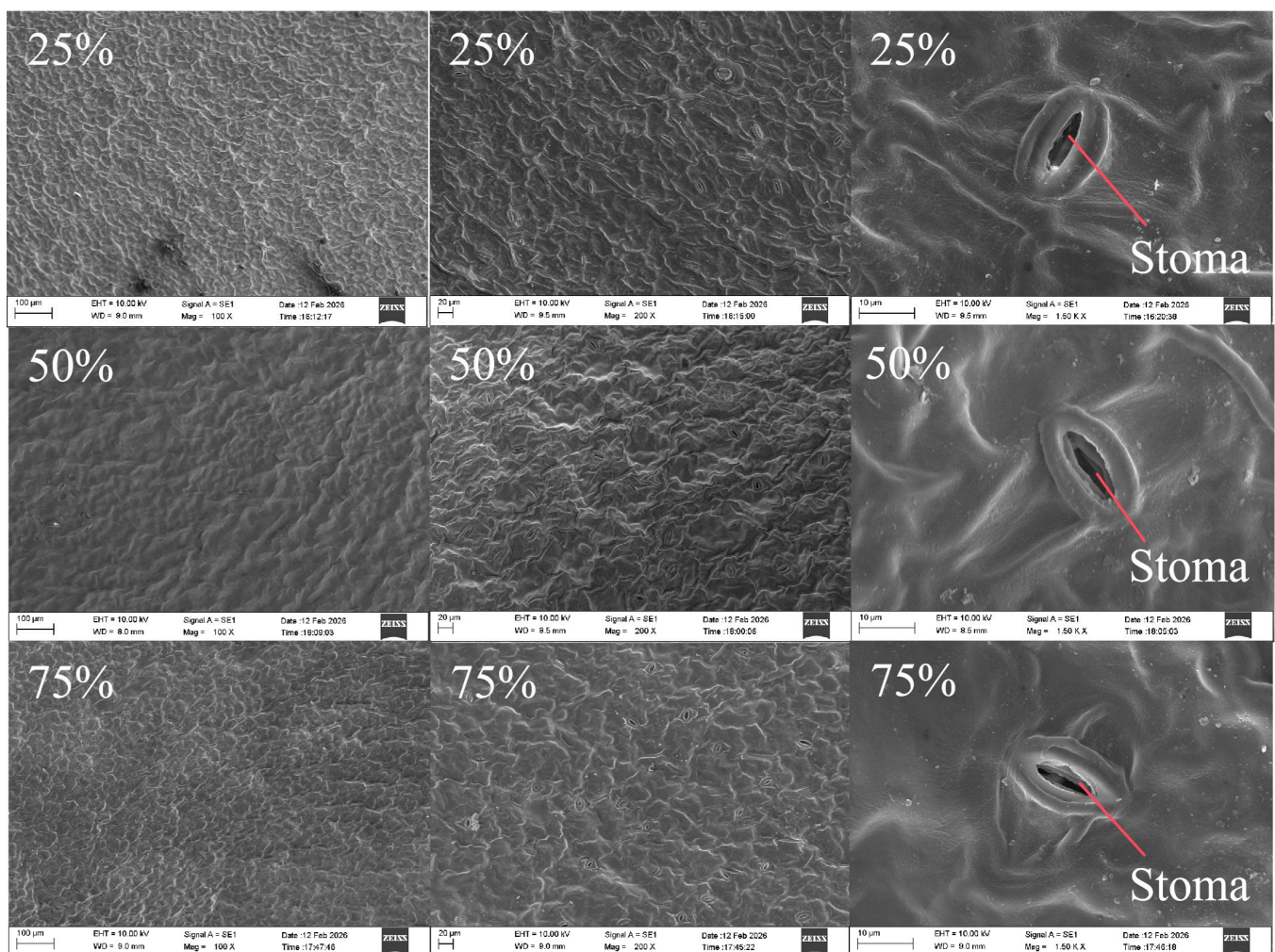


Figure 7. Leaf epidermal characteristics under different shading treatments. Images show representative leaf epidermal observations from the 25%, 50%, and 75% shade treatments. Scale bars and magnification are shown in each panel. Guard cells, stomatal pores, and surrounding epidermal cells are indicated by labels or arrows.

Among the leaf epidermal traits, SD had a markedly higher plasticity index than the other stomatal morphology traits, with a value of approximately 0.54. SL, SW, and SA had low plasticity indices of approximately 0.04, 0.11, and 0.09, respectively. The plasticity of stomatal traits followed the order SD > SW > SA > SL. Among the chlorophyll-related traits, Chl b had the highest plasticity index (approximately 0.82), followed by Chl(a+b) and Chl a (approximately 0.75 and 0.72, respectively). The plasticity index of Car was approximately 0.57, and that of Chl a/Chl b was approximately 0.42. The plasticity of chlorophyll-related traits followed the order Chl b > Chl(a+b) > Chl a > Car > Chl a/Chl b.

3.6. Correlation Analysis

As shown in Figure 11, the functional traits of *H. hainanensis* seedlings under different shade conditions showed correlations of varying strength. Shade level was significantly correlated with several growth traits. Shade level was significantly or highly significantly correlated with seedling height, ground diameter, leaf width, aboveground fresh weight, aboveground dry weight, root length, belowground fresh weight, and belowground dry

weight. Relatively strong correlations were observed for seedling height, ground diameter, aboveground biomass, and belowground biomass.

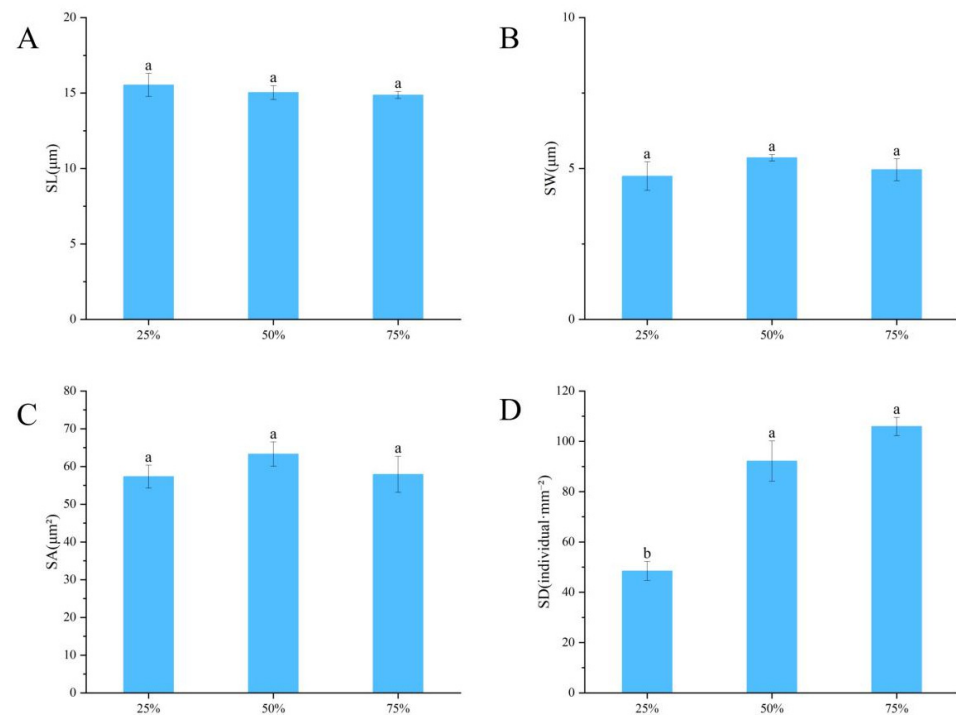


Figure 8. Stomatal traits of *Horsfieldia hainanensis* seedlings under different shading treatments. **(A)** SL, stomatal length; **(B)** SW, stomatal width; **(C)** SA, stomatal area; **(D)** SD, stomatal density. Units: SL and SW, μm ; SA, μm^2 ; SD, stomata mm^{-2} . Values are means $\pm$ SE. Different lowercase letters indicate significant differences among shade treatments at $p < 0.05$. Stomatal structures used for the measurements are shown in Figure 7.

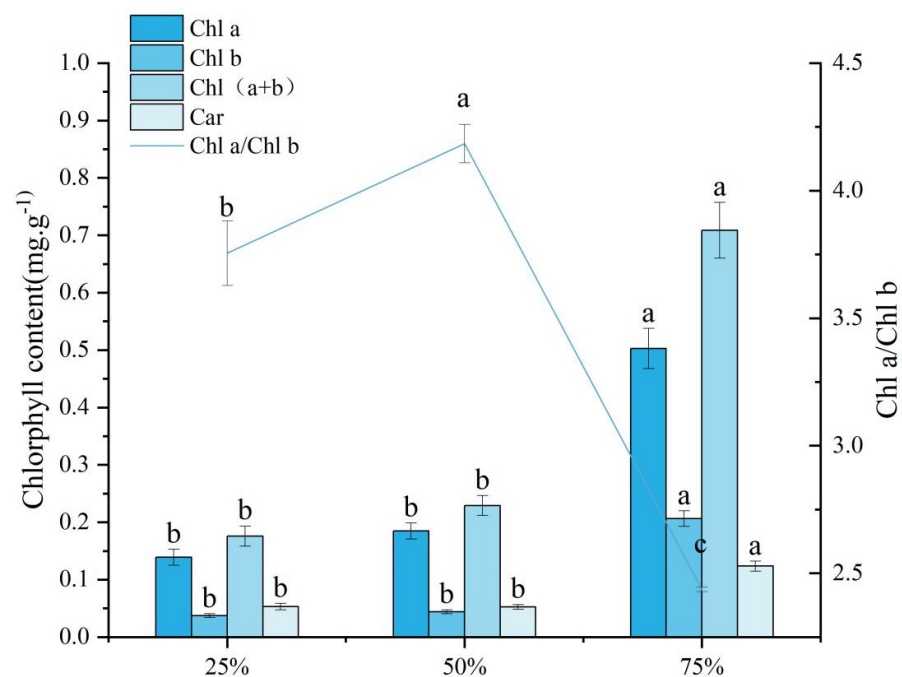


Figure 9. Photosynthetic pigment contents in the leaves of *Horsfieldia hainanensis* seedlings under different shading treatments. Note: Chl a, chlorophyll a content; Chl b, chlorophyll b content; Chl(a+b), total chlorophyll content; Car, carotenoid content; Chl a/Chl b, ratio of chlorophyll a to chlorophyll b. Different lowercase letters indicate significant differences among shade treatments for the same trait at $p < 0.05$.

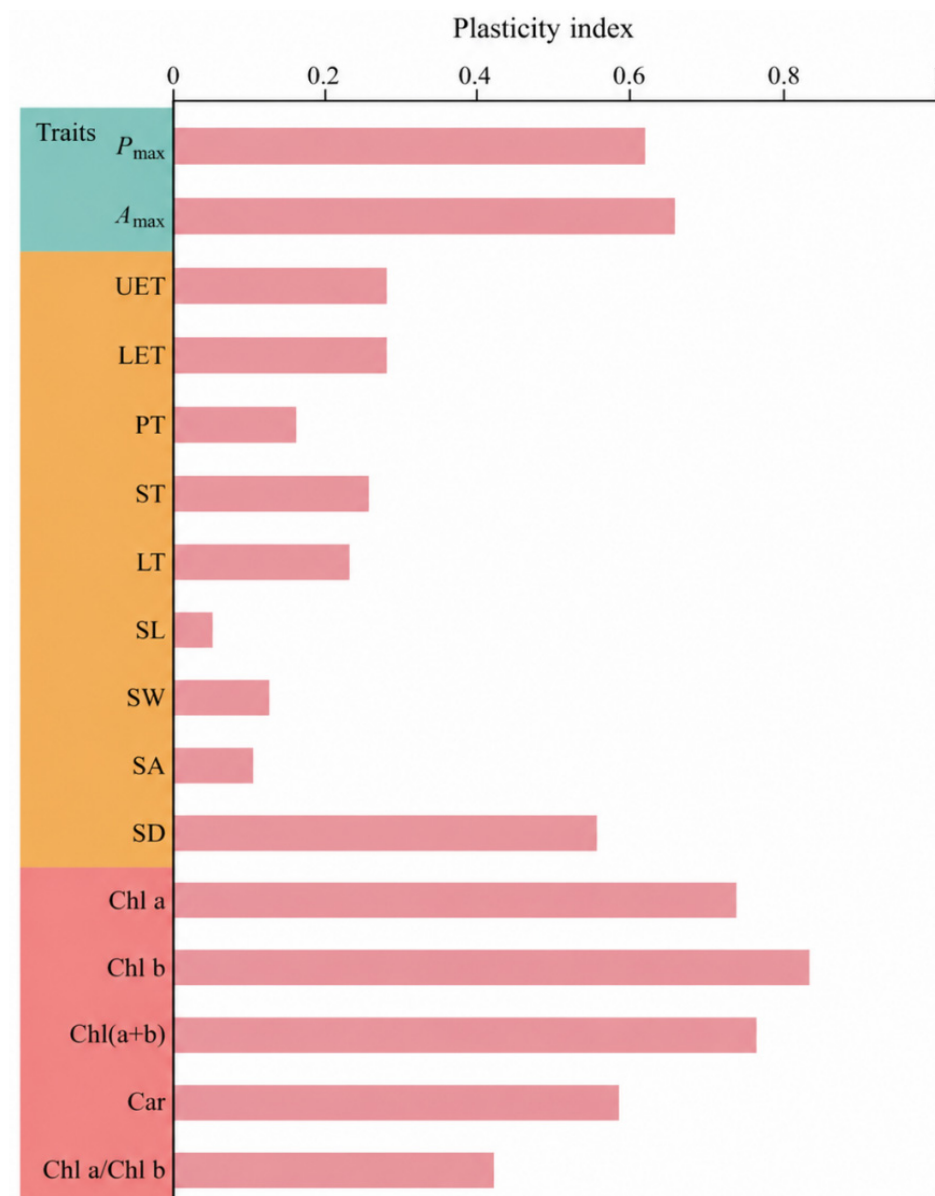


Figure 10. Plasticity index of functional traits of *Horsfieldia hainanensis* seedlings under different shading treatments.

Growth traits were generally strongly correlated. Seedling height, ground diameter, leaf width, aboveground fresh weight, aboveground dry weight, belowground fresh weight, and belowground dry weight were mostly significantly or highly significantly positively correlated. Correlations were high between aboveground fresh and dry weights and between belowground fresh and dry weights. Root length was also positively correlated with belowground fresh and dry weights. Leaf number and leaf length showed relatively weak correlations with other growth traits. Photosynthetic parameters were also clearly correlated. P_{max} was significantly positively correlated with A_{max} , and both parameters were positively correlated with several growth and biomass traits. LSP, LCP, CDSP, and CDCP showed alternating negative and positive correlations with some growth and photosynthetic capacity traits; LSP and LCP were mostly negatively correlated with P_{max} and A_{max} . R_d and R_p were also significantly correlated with several photosynthetic parameters. Leaf anatomical traits showed certain correlations with growth and photosynthetic traits. UET, PT, ST, and LT were mostly positively correlated with seedling height, ground diameter, leaf width, aboveground fresh weight, aboveground dry weight, belowground

fresh weight, and belowground dry weight. LT was positively correlated with UET, PT, and ST. PT/ST showed relatively weak correlations with most traits, with both positive and negative correlations observed. Among the stomatal traits, SL, SW, and SA were strongly and mostly significantly or highly significantly positively correlated. SD was significantly correlated with some growth traits, photosynthetic parameters, leaf anatomical traits, and chlorophyll traits. Compared with SL, SW, and SA, SD showed stronger correlations with other functional traits. Chlorophyll-related traits were strongly correlated with one another. Chl a, Chl b, Chl(a+b), and Car were mostly significantly or highly significantly positively correlated, with particularly high correlations between Chl a and Chl(a+b), between Chl b and Chl(a+b), and between Car and Chl(a+b).

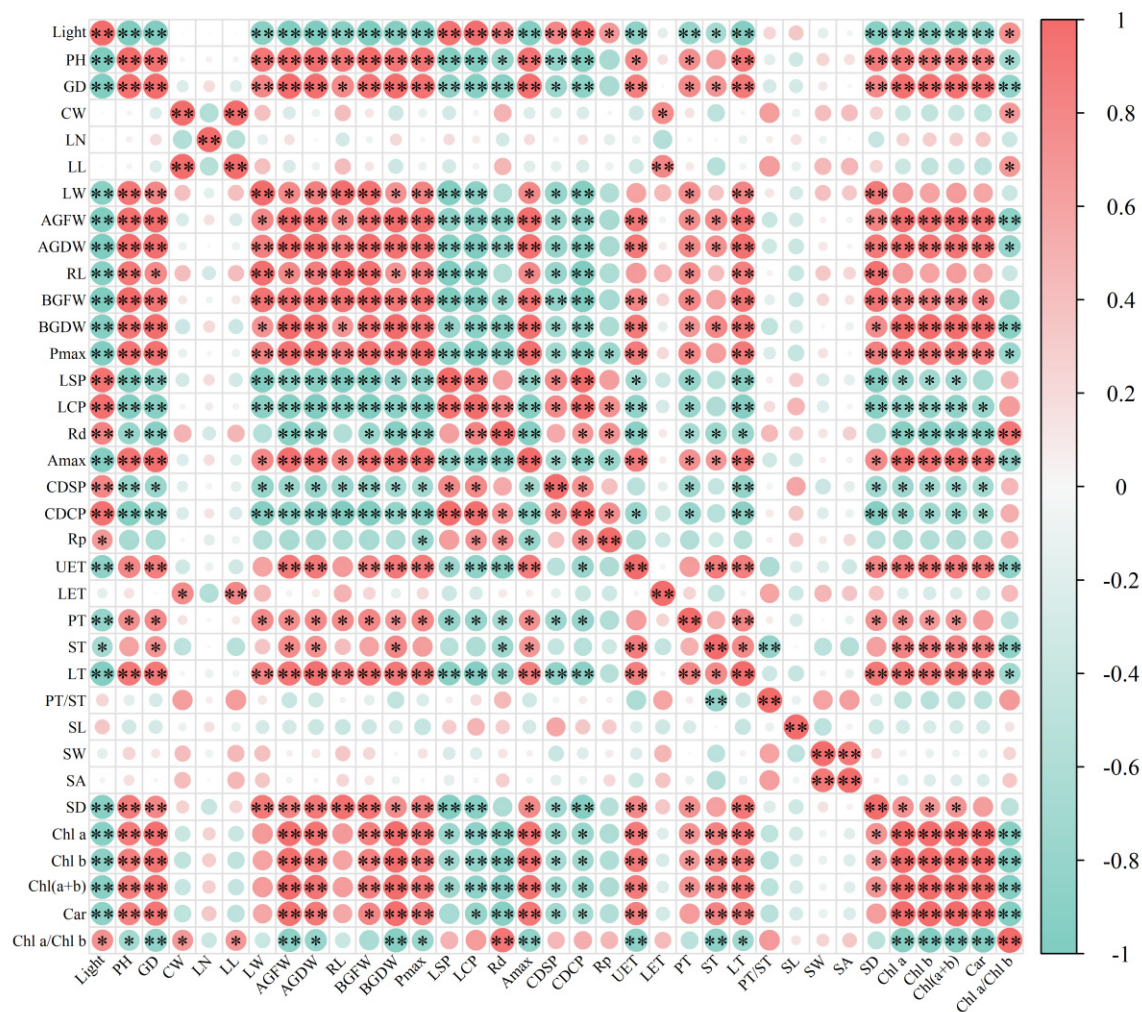


Figure 11. Pearson correlation analysis of functional traits of *Horsfieldia hainanensis* seedlings under different shading treatments. Note: PH, plant height; GD, ground diameter; CW, crown width; LN, leaf number; LL, leaf length; LW, leaf width; AGFW, aboveground fresh weight; AGDW, aboveground dry weight; RL, root length; BGFw, belowground fresh weight; BGDW, belowground dry weight. *, ** indicate significant correlations at $p < 0.05$ and $p < 0.01$, respectively.

4. Discussion

4.1. Growth Responses to Shade

Shade conditions significantly affect plant growth traits. Seedling height, ground diameter, crown width, and biomass are direct indicators of plant growth status and important criteria for evaluating the suitability of shade treatments [43]. Previous studies have shown that moderate shade can promote seedling growth and improve photosynthetic

traits [44]. In this study, all morphological growth traits followed the order 75% shade > 50% shade > 25% shade within the tested surviving shade range, indicating that growth improved with increasing shade level from 25% to 75%. This finding is generally consistent with previous studies. As a tropical rainforest understory species, *H. hainanensis* seedlings showed clear low-light acclimation. Excessively strong light may induce photoinhibition and photosystem damage, thereby suppressing normal seedling growth [45], whereas suitable shade may create a favorable light environment for leaf expansion, mesophyll tissue development, and dry matter accumulation.

Aboveground and belowground fresh and dry weights increased significantly with increasing shade, and the highest aboveground and belowground dry matter accumulation occurred under 75% shade. This result is consistent with the highest maximum net photosynthetic rate observed under the same treatment. Photosynthetic carbon fixation is the direct driving force for plant dry matter accumulation; enhanced photosynthetic capacity increases carbon-source supply and promotes organic matter allocation among organs [46]. Xu et al. (2024) also reported, in a comparative study of *H. hainanensis* seedlings and adult plants, that suitable shade and adequate water supply should be provided during the seedling stage and that shade intensity should be gradually reduced as plants grow [47]. This recommendation is highly consistent with the conservation implication of the present study.

Seedling quality should be evaluated comprehensively rather than by plant height alone. The coordinated increases in shoot growth, root development, and biomass under 75% shade indicate the production of more vigorous and balanced seedlings. In contrast, poor growth under 25% shade and severe wilting or mortality under full light suggest that excessive irradiance is unsuitable for early seedling cultivation. However, because shade levels above 75% and intermediate shade levels between 50% and 75% were not tested, 75% shade should be interpreted as the best-performing treatment among the surviving shade treatments tested, rather than as a universal optimum.

4.2. Photosynthetic Responses to Shade

Light is an essential environmental factor driving photosynthesis, and either excessive or insufficient light can adversely affect plant growth [48,49]. Previous work showed that the net photosynthetic rate of *Cremastra appendiculata* seedlings peaked in a weak-light environment under 80% shade [50]. Unsuitable light intensity can induce photoinhibition or insufficient light-use efficiency, thereby limiting biomass accumulation and plant growth. In this study, photosynthetic rate in *H. hainanensis* seedlings generally followed the order 75% shade > 50% shade > 25% shade. Under 75% shade, P_{max} ($4.19 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and A_{max} ($9.53 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) were significantly higher than those under the other treatments. By contrast, the 25% shade treatment had the highest LSP ($1234.56 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) but the lowest P_{max} and A_{max} . This indicates that stronger light does not necessarily enhance photosynthetic efficiency. Under lower shade, seedling photosynthetic potential was restricted, possibly because of reduced light-use efficiency or increased photoinhibition risk under strong light, a pattern also observed in several shade-tolerant species [50,51].

LSP and CDSP also reflect the ability of plants to use high irradiance and CO_2 [52,53]. In this study, with increasing shade, AQY increased from 0.026 to 0.050, LCP decreased from 23.92 to $8.27 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and R_d also decreased. A low LCP and a high AQY are generally considered typical response-curve characteristics of shade-acclimated plants. In this study, the lower LCP and higher AQY observed under 75% shade were consistent with the superior light-response performance of seedlings under relatively high shade. This pattern is consistent with the findings of Chai et al. (2018) in the shade-tolerant

plant *Camellia nitidissima*, in which AQY decreased and LCP increased with increasing light intensity [51]. The results further support the shade-adapted photosynthetic characteristics of *H. hainanensis* seedlings.

The CO₂-response parameters also showed that α and Amax increased significantly with increasing shade, whereas CDCP decreased from 147.27 to 39.46 $\mu\text{mol mol}^{-1}$. The lower CDCP under higher shade indicates that the fitted Pn–Ci curve reached positive net photosynthesis at a lower CO₂ concentration, suggesting a more favorable CO₂-response pattern under the measurement conditions. The CO₂-response parameters showed that, with increasing shade, α and Amax in *H. hainanensis* seedlings increased significantly, whereas CDCP and CDSP generally decreased. Under 75% shade, both Amax and α reached their highest values, while CDCP decreased to the lowest level. These results indicate that seedlings under relatively high shade had a stronger response to changes in CO₂ availability and were able to maintain positive net photosynthesis at lower CO₂ concentrations. Together with the higher AQY and Pmax and the lower LCP under 75% shade in the light-response curves, this pattern suggests that this treatment was more favorable for maintaining carbon assimilation under low-light conditions. Meanwhile, seedlings under 75% shade also showed the best overall performance in growth, leaf development, and biomass accumulation, indicating consistency between photosynthetic performance and seedling establishment. Previous studies have shown that growth irradiance can markedly affect photosynthetic response characteristics in plants [54,55]. Combined with the present results, relatively high shade appears to better match the ecological requirements of *H. hainanensis* seedlings, and 75% shade can be considered a suitable light condition for nursery cultivation and early establishment. However, biochemical and diffusion-related mechanisms such as Rubisco activity, Vcmax, Jmax, stomatal conductance, and mesophyll conductance were not directly measured in this study. Therefore, these mechanisms cannot be confirmed from the present data and should be considered only as possible explanations based on previous literature. Future studies combining gas-exchange curves with chlorophyll fluorescence, Rubisco activity, and mesophyll conductance measurements are needed to clarify the biochemical basis of photosynthetic acclimation in *H. hainanensis* seedlings.

Based on the light- and CO₂-response parameters, *H. hainanensis* seedlings showed the highest photosynthetic carbon assimilation capacity under 75% shade within the effective shade gradient tested. This conclusion is consistent with the comparative study by Xu et al. (2024), which reported that the photosynthetic system in *H. hainanensis* is less developed at the seedling stage than at the adult stage and that seedlings are more suitable for growth under relatively high shade [47]. Photosynthetic parameters can serve as useful indicators of nursery light suitability. Higher Pmax, Amax, and AQY, together with lower LCP and CDCP under 75% shade, indicate enhanced carbon assimilation and efficient utilization of low light. These traits support biomass accumulation and seedling establishment and can provide a physiological basis for optimizing shade management and pre-transplant acclimation.

4.3. Leaf Microstructural Responses to Shade

Leaf anatomical differentiation is an integrated morphological expression of plant responses to light environments. In many species, leaves formed under shade are often thinner than those exposed to higher light; therefore, the increase in leaf thickness (LT), palisade parenchyma thickness (PT), and spongy parenchyma thickness (ST) with increasing shade observed in this study represents a noteworthy anatomical response. This pattern may be related to several non-exclusive factors. First, the relatively thinner leaves under 25% shade may reflect stronger light stress or restricted leaf development during early seedling growth. Second, as a tropical rainforest understory species, *H. hainanensis* seedlings may

maintain more developed mesophyll tissues under shaded nursery conditions. Third, differences in leaf developmental stage, sampling position, or individual seedling variation may also have contributed to the observed anatomical pattern. Therefore, the increase in LT, PT, and ST under stronger shade should be interpreted as an observed structural response under the present nursery conditions rather than as a fully confirmed mechanism of shade adaptation.

Leaf thickness (LT), palisade parenchyma thickness (PT), and the PT/ST ratio are closely related to photosynthetic efficiency [56]. In this study, LT, UET, PT, and ST increased with increasing shade, and mesophyll tissue was most fully developed under 75% shade. Previous studies have reported associations between leaf tissue thickness, mesophyll organization, chlorophyll accumulation, and photosynthetic traits under different light environments [57]. This pattern corresponded well with the observed increase in chlorophyll content under stronger shade, indicating that *H. hainanensis* seedlings can improve low-light acclimation by adjusting leaf anatomical structure and showing coordinated morphological and functional responses. The PT/ST ratio did not differ significantly among shade treatments and was only slightly higher under 50% shade, indicating that the relative proportions of palisade and spongy tissues remained largely stable. Shade-tolerant plants in low-light environments are generally considered to rely more on spongy tissue development and intercellular-space adjustment to improve internal CO₂ diffusion than solely on palisade tissue thickening [56]. In this study, the plasticity index of PT (0.15) was lower than that of ST (0.23), indicating that the structural plasticity of palisade tissue was relatively weak and that structural responses to shade were more evident in spongy tissue. This feature may be associated with internal gas-exchange conditions and is consistent with the ecological habit of this species in shaded understory habitats. Studies on light-environment acclimation in endangered *Heptacodium miconioides* seedlings have also shown that light intensity significantly affects leaf morphology, anatomical structure, photosynthetic traits, and chloroplast ultrastructure, suggesting that plants can adapt to different light environments through coordinated adjustments of leaf structure and photosynthetic systems [49].

Many studies have shown that stomatal development is regulated by light [58] and that stomatal density is strongly influenced by light intensity [59]. In this study, shade treatment did not significantly affect SL, SW, or SA, indicating that individual stomatal morphology was relatively stable under shade. By contrast, SD was more sensitive to shade. SD under 25% shade was significantly lower than that under 50% and 75% shade, suggesting that the observed epidermal response was mainly reflected in stomatal number per unit leaf area rather than in individual stomatal morphology. Previous studies have reported that, in woody plants under different light environments, stomatal size often changes only slightly, whereas stomatal density and stomatal index show higher plasticity. For example, Aasamaa and Aphalo found that shade acclimation in *Tilia cordata* Mill. was not strongly dependent on changes in stomatal size but was more evident in adjustments of stomatal density and stomatal index [60]. Stomata are key regulators of CO₂ uptake and water loss, and changes in stomatal number can affect potential gas-exchange capacity and water-use strategy [61]. The reduced stomatal density under the relatively high-light 25% shade treatment may represent a regulatory strategy to reduce potential transpirational water loss and maintain leaf water balance. By contrast, maintaining higher stomatal density under 50% and 75% shade may help secure CO₂ supply under weak light and support higher net photosynthetic rates. The plasticity index of SD (0.54) was higher than those of SL (0.04), SW (0.11), and SA (0.09), showing a greater PPI value for stomatal density than for stomatal size-related traits [62].

4.4. Chlorophyll Responses to Shade

The results showed that Chl a, Chl b, Chl(a+b), and Car contents in *H. hainanensis* seedling leaves increased significantly with increasing shade, and all pigment contents under 75% shade were clearly higher than those under 25% shade. This indicates that, under stronger shade, *H. hainanensis* seedlings enhanced weak-light capture by increasing photosynthetic pigment accumulation, which is an important physiological adjustment for acclimation to low-light environments. Similar patterns have been observed in many other plant species [63,64]. Previous studies have shown that shade can promote chlorophyll and carotenoid accumulation in leaves, thereby improving light absorption and utilization. In woody seedlings, suitable shade can promote chlorophyll accumulation and thereby enhance light harvesting under low-light conditions [65]. In this study, the increase in pigment contents under higher shade was consistent with the greater light-capture capacity of *H. hainanensis* seedlings. Because light-harvesting complex activity and leaf optical absorption were not directly measured in this study, this interpretation should be regarded as an inference based on pigment accumulation and photosynthetic response parameters. Therefore, the increase in pigment contents under weak light may represent a shade-associated physiological response of *H. hainanensis* seedlings to nursery shade conditions.

In addition, Chl b increased more strongly than Chl a under 75% shade, resulting in a lower Chl a/Chl b ratio under high shade. A lower Chl a/Chl b ratio generally indicates a relatively higher proportion of light-harvesting complexes (LHCs), which helps improve weak-light capture per unit leaf area and is an important physiological characteristic of shade-tolerant plants [66]. Plasticity analysis showed that the plasticity index of Chl b (0.82) was higher than those of Chl a (0.72) and Car (0.57), suggesting that Chl b was relatively more responsive to shade variation and may be associated with adjustments in pigment composition under shaded conditions, rather than with a uniform increase in all pigment components. This is consistent with the findings of Khan et al. (2023) in tomato, in which increased Chl b accumulation improved growth under low-light conditions and enhanced light-use capacity by reducing the Chl a/Chl b ratio [67]. Dziwulska-Hunek et al. (2025) also reported that changes in leaf pigment composition were closely associated with plant acclimation to the light environment in perennial leguminous plants [68].

In this study, seedlings grew better under the 75% shade treatment than under the 25% and 50% shade treatments, suggesting that relatively high shade within the tested range may be more suitable for *H. hainanensis* during the seedling stage. Pigment regulation under this treatment reflected typical understory low-light acclimation. The significant increase in Car content under 75% shade also suggests that carotenoid accumulation changed together with chlorophyll accumulation under the higher shade treatment, which may reflect a coordinated shift in pigment composition. Overall, the results support a staged light-management strategy for *H. hainanensis* seedlings. Approximately 75% shade is optimal during nursery cultivation, whereas a gradual reduction in shade before outplanting may improve acclimation to natural light conditions. For restoration or ex situ cultivation, partially shaded habitats or temporary artificial shading are likely more suitable than fully exposed sites during early establishment. Nevertheless, field transplantation, gradual shade reduction, acclimation to natural light, and post-transplant survival were not evaluated in the present study. Therefore, the practical application of this shade level in field reintroduction or cultivation should be further validated under field or semi-natural conditions. These findings provide a practical basis for artificial propagation, nursery management, seedling-quality evaluation, and field establishment of *H. hainanensis*.

5. Conclusions

The results showed that different shade treatments significantly affected the functional traits of *H. hainanensis* seedlings. Seedlings under 0% shade showed severe wilting and mortality, indicating that they were not suited to long-term exposure to full-light conditions under the present experimental conditions. The 75% shade treatment showed higher Pmax, AQY, α , and Amax, together with lower LCP and CDCP, indicating better light- and CO₂-response performance based on the fitted response-curve parameters. Leaf microstructure responded clearly to shade variation. Within the 25–75% shade range, leaf thickness, epidermal thickness, palisade tissue thickness, and spongy tissue thickness generally increased with increasing shade level, indicating that leaf microstructure showed clear responses to the tested shade conditions. The palisade-to-spongy tissue ratio changed only slightly among treatments, indicating that internal leaf tissue proportions remained relatively stable. Stomatal density was sensitive to shade variation, whereas stomatal length, width, and area changed only slightly, indicating that the stomatal response of *H. hainanensis* seedlings to shade was mainly expressed through quantitative adjustment. Changes in chlorophyll composition indicate that *H. hainanensis* seedlings can improve light capture under low-light conditions by adjusting photosynthetic pigment contents and their proportions. Correlation analysis showed that seedling height, ground diameter, leaf width, aboveground fresh weight, aboveground dry weight, belowground fresh weight, and belowground dry weight were mostly positively correlated, with many of these correlations reaching statistical significance. In contrast, LSP, LCP, CDSP, and CDCP were generally negatively correlated with Pmax, Amax, and major growth traits, suggesting that lower compensation and saturation thresholds were associated with higher photosynthetic-capacity parameters and better growth performance under the tested shade conditions.

Therefore, under the tested nursery shade-net conditions, the approximately 75% shade treatment may be suitable for the artificial propagation and nursery cultivation of *H. hainanensis* seedlings. Because field transplantation, gradual hardening, natural-light acclimation, and post-planting survival were not examined, the suitability of the 75% shade regime for field establishment or reintroduction requires further validation. Future studies should integrate field transplantation, post-planting survival, substrate, water availability, fertilization, and shade-transition treatments to optimize cultivation and establishment strategies for this species.

Author Contributions: L.G.: Writing—original draft, Software, Investigation, Formal analysis. S.S.: Investigation, Conceptualization. X.L.: Investigation, Conceptualization. H.W.: Investigation, Conceptualization. C.Z.: Writing—review and editing, Software, Resources, Funding acquisition. L.L.: Investigation, Formal analysis. J.T.: Resources, Conceptualization. R.Z.: Investigation, Conceptualization. X.W.: Investigation, Formal analysis. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Nature Reserve Project (GK2622) of the Central Finance Forestry and Grassland Ecological Protection and Restoration Fund; the Basic Research Business Expense Support Project of Guangxi Institute of Botany (Guizhiye 25003); the National Natural Science Foundation of China (32560330); the Guangxi Natural Science Foundation (Project Nos. 2024GXNSFAA010452 and 2023GXNSFAA026253); the Autonomous Projects of the Guangxi Key Laboratory of Functional Phytochemicals Research and Sustainable Utilisation (Project Nos. ZRJ2024-3 and ZRJ2024-11); the Hechi Municipal Science and Technology Base and Talent Programme (HeKe AC231113); and the 2022 Guilin Municipal Technology Application and Promotion Programme (20220134-3 and 20230102-3).

Data Availability Statement: The data presented in this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Liu, S.; Mei, W.; Zeng, Y.; Wang, H.; Li, X.; Dai, H. Study on liposoluble constituents of the Li medicine *Horsfieldia hainanensis*. *Chin. J. Ethnomedicine Ethnopharmacology* **2010**, *19*, 147–149. (In Chinese) [[CrossRef](#)]
2. Zuo, J.; Yang, T.; Qin, Y.; Pan, W.; Chen, B.; Yang, S.; Li, W. Analysis of fruit traits of *Horsfieldia hainanensis*. *Territ. Nat. Resour. Study* **2025**, *5*, 88–91. (In Chinese) [[CrossRef](#)]
3. Dang, J.; Yang, X.; Huang, Y.; Ye, F.; Luo, T.; Chen, S.; Liu, B.; Bai, Y. GC–MS analysis of chemical constituents of volatile oil from the bark of *Horsfieldia hainanensis*. *J. Chin. Med. Mater.* **2009**, *32*, 714–716. (In Chinese) [[CrossRef](#)]
4. Liang, H.; Lin, Y.; Liu, X.; Jiang, Y.; Yang, J.; Huang, R.; Wang, R.; Wang, Y. Habitat suitability evaluation of the endangered plant *Horsfieldia hainanensis* based on an optimized MaxEnt model. *J. Cent. South Univ. For. Technol.* **2024**, *44*, 16–26. (In Chinese) [[CrossRef](#)]
5. Jiang, Y.; Liu, X.; Jiang, Y.; He, Y.; Xiang, W. Anatomical characteristics of vegetative organs of the endangered plant *Horsfieldia hainanensis*. *Guihaia* **2018**, *38*, 843–850. (In Chinese) [[CrossRef](#)]
6. Xu, J.; Wei, X.; Zou, R. Research progress on the plant species with extremely small populations *Horsfieldia hainanensis*. *South-Cent. Agric. Sci. Technol.* **2024**, *45*, 234–236. (In Chinese)
7. Fu, G. Strategies for the development and utilization of medicinal plant resources in tropical rain forests of Hainan Island. *Trop. For.* **2004**, *1*, 6–7. (In Chinese) [[CrossRef](#)]
8. He, G.; Cai, L.; Liang, G.; Shi, Q.; Chen, L. Seedling-raising techniques for *Horsfieldia hainanensis* by sowing. *Pract. For. Technol.* **2013**, *6*, 35–37. (In Chinese) [[CrossRef](#)]
9. Huang, J.; Wang, H.; Zhong, Y.; Huang, J.; Fu, X.; Wang, L.; Teng, W. Growth and physiological response of an endangered tree, *Horsfieldia hainanensis* Merr., to simulated sulfuric and nitric acid rain in southern China. *Plant Physiol. Biochem.* **2019**, *144*, 118–126. [[CrossRef](#)] [[PubMed](#)]
10. Liu, X.; Xiao, Y.; Wang, Y.; Wang, R.; Huang, R.; Liang, H.; Jiang, Y.; Jiang, Y. Seed germination ecology of endangered plant *Horsfieldia hainanensis* Merr. in China. *BMC Plant Biol.* **2024**, *24*, 486. [[CrossRef](#)] [[PubMed](#)]
11. Hu, Q.; Kang, Y.; Zhang, B.; Wang, L.; Teng, W. Growth response and comprehensive evaluation of *Horsfieldia hainanensis* under lead and cadmium stress. *Guangxi For. Sci.* **2019**, *48*, 202–206. (In Chinese)
12. Dang, J.; Yang, X.; Chen, S.; Luo, T.; Ye, F.; Huang, Y.; Bai, Y.; Liu, B. GC–MS analysis of chemical constituents of volatile oil from leaves of *Horsfieldia hainanensis*. *Lishizhen Med. Mater. Medica Res.* **2009**, *20*, 2188–2189. (In Chinese)
13. Xu, J.; Wei, X.; Tang, J.; Zou, R. Optimization of total flavonoid extraction from *Horsfieldia hainanensis* by response surface methodology and antioxidant activity. *Guangxi Sci.* **2024**, *31*, 712–722. (In Chinese)
14. Xu, J.; Wei, X.; Tang, J.; Zou, R. Optimization of total saponin extraction from *Horsfieldia hainanensis* by response surface methodology and antioxidant activity. *China Feed.* **2025**, *9*, 28–37. (In Chinese) [[CrossRef](#)]
15. Jiang, Y.H.; Xiang, W.H.; Jiang, Y.; He, Y.H.; Lin, J.Y. Floristic composition, structure and phytogeographic characteristics of the *Horsfieldia hainanensis* community in Guangxi. *J. Beijing For. Univ.* **2016**, *38*, 74–82. (In Chinese) [[CrossRef](#)]
16. Jiang, Y.; Xiang, W.; He, Y.; Jiang, Y.; Lin, J.; Huang, R. Quantitative characteristics and dynamics of the *Horsfieldia hainanensis* population with extremely small population size. *J. Cent. South Univ. For. Technol.* **2017**, *37*, 66–71, 80. (In Chinese) [[CrossRef](#)]
17. Xu, Y.; Luo, W.; Han, W.; Peng, H. Preliminary survey of wild plant species with extremely small populations in Bawangling, Hainan Island. *Trop. For.* **2014**, *42*, 32–36. (In Chinese) [[CrossRef](#)]
18. Cai, C.N.; Ma, H.; Ci, X.Q.; Conran, J.G.; Li, J. Comparative phylogenetic analyses of Chinese *Horsfieldia* (Myristicaceae) using complete chloroplast genome sequences. *J. Syst. Evol.* **2021**, *59*, 504–514. [[CrossRef](#)]
19. Xu, J.; Zou, R.; Chai, S.; Jiang, H.; Zhong, W.; Tang, J.; Wei, X. Single Nucleotide Polymorphism Markers Based on Hyper-Seq Revealed the Genetic Diversity and Genetic Relationship of *Horsfieldia hainanensis* in China. *Forests* **2025**, *16*, 217. [[CrossRef](#)]
20. Jiang, Y.; Liu, X.; Xiang, W.; Jiang, Y.; He, Y. Genetic diversity and structure analysis of the endangered plant species *Horsfieldia hainanensis* Merr. in China. *Biotechnol. Biotechnol. Equip.* **2018**, *32*, 95–101. [[CrossRef](#)]
21. Cai, C.; Hou, Q.; Ci, X.; Xiao, J.; Zhang, C.; Li, J. Genetic diversity of the plant species with extremely small populations *Horsfieldia hainanensis*. *J. Trop. Subtrop. Bot.* **2021**, *29*, 547–555. (In Chinese) [[CrossRef](#)]
22. Wang, X.; Chen, G.; Du, S.; Wu, H.; Fu, R.; Yu, X. Light intensity influence on growth and photosynthetic characteristics of *Horsfieldia hainanensis*. *Front. Ecol. Evol.* **2021**, *9*, 636804. [[CrossRef](#)]
23. Fu, X.; Wang, L.; Zhu, Y.; Xu, Q.; Wang, L.; Teng, W. Effects of different fertilization treatments on the growth and physiological characteristics of *Horsfieldia hainanensis* seedlings. *Plant Sci. J.* **2018**, *36*, 273–281. (In Chinese) [[CrossRef](#)]
24. Fan, R.Y.; Yang, J.S.; Wang, R.J.; Jiang, Y.; Li, J.; Jiang, Y.; Huang, R.L.; Liu, X.S. Response of seed germination to high temperature and humidity of the wild plant species with extremely small populations, *Horsfieldia hainanensis*. *Guihaia* **2026**, *46*, 75–85. (In Chinese) [[CrossRef](#)]

25. Luo, W.J.; Wang, Y.J.; Wang, L.H. Irradiation and nitrogen regulate growth and physiology in *Horsfieldia hainanensis* seedlings. *Biol. Plant.* **2023**, *67*, 19–27. [[CrossRef](#)]
26. Tang, W.; Guo, H.; Baskin, C.C.; Xiong, W.; Yang, C.; Li, Z.; Song, H.; Wang, T.; Yin, J.; Wu, X.; et al. Effect of light intensity on morphology, photosynthesis and carbon metabolism of alfalfa (*Medicago sativa*) seedlings. *Plants* **2022**, *11*, 1688. [[CrossRef](#)] [[PubMed](#)]
27. Wang, R.; Ma, J.; Huang, R.; Wang, Y.; Jiang, Y.; Ling, Y.; Yang, J.; Liang, H.; Liu, X.; Liao, N. The effects of shading on the photosynthetic performance of endangered plant *Horsfieldia hainanensis* seedlings. *Forests* **2024**, *15*, 3. [[CrossRef](#)]
28. He, D.; Li, H.; Zhou, P.; Guo, J.; Yuan, J.; Wang, J.; Liu, Y.; Zhang, Z.; Lei, Y. Leaf plasticity responses of four urban garden plants to low-light environments under viaducts. *Forests* **2025**, *16*, 651. [[CrossRef](#)]
29. Kong, D.X.; Li, Y.Q.; Wang, M.L.; Bai, M.; Zou, R.; Tang, H.; Wu, H. Effects of light intensity on leaf photosynthetic characteristics, chloroplast structure, and alkaloid content of *Mahonia bodinieri* (Gagnep.) Laferr. *Acta Physiol. Plant.* **2016**, *38*, 120. [[CrossRef](#)]
30. Yadav, A.; Singh, D.; Lingwan, M.; Yadukrishnan, P.; Masakapalli, S.K.; Datta, S. Light signaling and UV-B-mediated plant growth regulation. *J. Integr. Plant Biol.* **2020**, *62*, 1270–1292. [[CrossRef](#)] [[PubMed](#)]
31. Wei, Y.; Wang, S.; Yu, D. The role of light quality in regulating early seedling development. *Plants* **2023**, *12*, 2746. [[CrossRef](#)] [[PubMed](#)]
32. Bao, Y.; Liu, X.; Feng, C.H.; Niu, M.X.; Liu, C.; Wang, H.L.; Yin, W.; Xia, X. Light and light signals regulate growth and development in woody plants. *Forests* **2024**, *15*, 523. [[CrossRef](#)]
33. Wu, W.; Chen, L.; Liang, R.; Huang, S.; Li, X.; Huang, B.; Luo, H.; Zhang, M.; Wang, X.; Zhu, H. The role of light in regulating plant growth, development and sugar metabolism: A review. *Front. Plant Sci.* **2025**, *15*, 1507628. [[CrossRef](#)] [[PubMed](#)]
34. Lu, D.; Xu, B.; Yu, Q.; Liu, Z.; Ren, M.; Wang, Y.; Zhang, S.; Wu, C.; Shen, Y. Identification of potential light deficiency response regulators in endangered species *Magnolia sinostellata*. *Sci. Rep.* **2022**, *12*, 22536. [[CrossRef](#)] [[PubMed](#)]
35. Chen, C.I.; Lin, K.H.; Lin, T.C.; Huang, M.Y.; Chen, Y.C.; Huang, C.C.; Wang, C.W. Responses of photosynthesis and chlorophyll fluorescence during light induction in different seedling ages of *Mahonia oiwakensis*. *Bot. Stud.* **2023**, *64*, 5. [[CrossRef](#)] [[PubMed](#)]
36. Wang, X.; Wu, H.; Fu, R.; Du, S.; Chen, G. Effects of different canopy closures and altitudes on the leaves and growth indexes of *Horsfieldia hainanensis* Merr. reintroduced. *J. Trop. Biol.* **2021**, *12*, 481–490. (In Chinese) [[CrossRef](#)]
37. Tang, J.; Qin, H.; Pan, Z.; Zou, R. Seedling quality grading of *Cycas* plants from Shiwandashan. *Henan Agric.* **2022**, *32*, 19–21. (In Chinese) [[CrossRef](#)]
38. Chen, T.; Gu, C.; Yu, H.; Yang, Y.; Tang, J.; Zou, R.; Wei, X. Seedling quality grading of *Garcinia paucineris*. *Guangdong Seric.* **2022**, *56*, 18–23. (In Chinese) [[CrossRef](#)]
39. Ye, Z. A review on modeling light and CO₂ responses of photosynthesis. *Chin. J. Plant Ecol.* **2010**, *34*, 727–740. (In Chinese) [[CrossRef](#)]
40. Pan, L.; Tang, J.; Chen, T.; Zhu, S.; Zou, R.; Wei, X. Comparison of leaf photosynthetic function and structure between seedlings and adult plants of *Kmeria septentrionalis*. *Guangxi Sci.* **2023**, *30*, 1163–1170. (In Chinese) [[CrossRef](#)]
41. Li, Z. *Plant Microtechnique*; Science Press: Beijing, China, 1978. (In Chinese)
42. Li, H. *Principles and Techniques of Plant Physiological and Biochemical Experiments*; Higher Education Press: Beijing, China, 2000. (In Chinese)
43. Guzman, A.Q.; Cordero, R.A.S.; Corea, E.A. Biomass allocation and gas exchange are affected by light conditions in endangered *Cedrela salvadorensis* (Meliaceae) seedlings. *Rev. DE Biol. Trop.* **2016**, *64*, 1143–1154. [[CrossRef](#)] [[PubMed](#)]
44. Naveed, M.; Bansal, U.; Kaiser, B.N. Impact of low light intensity on biomass partitioning and genetic diversity in a chickpea mapping population. *Front. Plant Sci.* **2024**, *15*, 1292753. [[CrossRef](#)] [[PubMed](#)]
45. Chen, H.; Wang, L.; Guo, S.; Li, M.; Tian, Z.; Han, B.; Tang, X.; Liu, B. Effects of light intensity on seedling emergence and early growth of *Liquidambar formosana* Hance. *Forests* **2023**, *14*, 867. [[CrossRef](#)]
46. Feng, L.; Raza, M.A.; Li, Z.; Chen, Y.; Khalid, M.H.B.; Du, J.; Liu, W.; Wu, X.; Song, C.; Yu, L.; et al. The influence of light intensity and leaf movement on photosynthesis characteristics and carbon balance of soybean. *Front. Plant Sci.* **2019**, *9*, 1952. [[CrossRef](#)] [[PubMed](#)]
47. Xu, J.; Tang, J.; Jiang, H.; Zou, R.; Wei, X. A comparative study on leaf anatomy and photosynthetic characteristics of different growth stages of *Horsfieldia hainanensis*. *PeerJ* **2024**, *12*, e18640. [[CrossRef](#)] [[PubMed](#)]
48. Wu, L.; Zhang, W.; Ding, Y.; Zhang, J.; Cambula, E.D.; Weng, F.; Liu, Z.; Ding, C.; Tang, S.; Chen, L.; et al. Shading contributes to the reduction of stem mechanical strength by decreasing cell wall synthesis in japonica rice (*Oryza sativa* L.). *Front. Plant Sci.* **2017**, *8*, 881. [[CrossRef](#)] [[PubMed](#)]
49. Zhang, Y.; Chen, C.; Jin, Z.; Yang, Z.; Li, Y. Leaf anatomy, photosynthesis, and chloroplast ultrastructure of *Heptacodium miconioides* seedlings reveal adaptation to light environment. *Environ. Exp. Bot.* **2022**, *195*, 104780. [[CrossRef](#)]
50. Liu, B.; Wang, S.; Li, J.; Wang, J.; Hou, X.; Zhang, Y.; Wang, L. Effects of different light intensities on the growth and photosynthetic physiological characteristics of *Cremastra appendiculata* (D. Don) Makino seedlings. *Plants* **2026**, *15*, 388. [[CrossRef](#)] [[PubMed](#)]

51. Chai, S.; Tang, J.; Mallik, A.; Shi, Y.; Zou, R.; Li, J.; Wei, X. Eco-physiological basis of shade adaptation of *Camellia nitidissima*, a rare and endangered forest understory plant of Southeast Asia. *BMC Ecol.* **2018**, *18*, 5. [[CrossRef](#)] [[PubMed](#)]
52. Zhou, J.; Li, P.; Wang, J. Effects of light intensity and temperature on the photosynthesis characteristics and yield of lettuce. *Horticulturae* **2022**, *8*, 178. [[CrossRef](#)]
53. Li, Y.H.; Zhang, K.M.; Yu, H.F. Photosynthetic characteristics of ten cultivars of autumn chrysanthemum (*Dendranthema morifolium*) and correlation analysis between net photosynthetic rate and some physio-ecological factors. *J. Plant Resour. Environ.* **2012**, *21*, 70–76. (In Chinese)
54. Tang, X.; Liu, G.; Jiang, J.; Lei, C.; Zhang, Y.; Wang, L.; Liu, X. Effects of growth irradiance on photosynthesis and photorespiration of *Phoebe bournei* leaves. *Funct. Plant Biol.* **2020**, *47*, 1053–1061. [[CrossRef](#)] [[PubMed](#)]
55. Taylor, S.H.; Gonzalez-Escobar, E.; Page, R.; Parry, M.A.J.; Long, S.P.; Carmo-Silva, E. Faster than expected Rubisco deactivation in shade reduces cowpea photosynthetic potential in variable light conditions. *Nat. Plants* **2022**, *8*, 118–124. [[CrossRef](#)] [[PubMed](#)]
56. Wei, C.; Luo, G.; Jin, Z.; Li, J.; Li, Y. Physiological and structural changes in leaves of *Platycrater arguta* seedlings exposed to increasing light intensities. *Plants* **2024**, *13*, 1263. [[CrossRef](#)] [[PubMed](#)]
57. Aneja, P.; Sanyal, R.; Ranjan, A. Leaf growth in third dimension: A perspective of leaf thickness from genetic regulation to ecophysiology. *New Phytol.* **2025**, *245*, 989–999. [[CrossRef](#)] [[PubMed](#)]
58. Casson, S.A.; Franklin, K.A.; Gray, J.E.; Grierson, C.S.; Whitelam, G.C.; Hetherington, A.M. Phytochrome B and PIF4 regulate stomatal development in response to light quantity. *Curr. Biol.* **2009**, *19*, 229–234. [[CrossRef](#)] [[PubMed](#)]
59. Hronkova, M.; Wiesnerova, D.; Simkova, M.; Skupa, P.; Dewitte, W.; Vrablova, M.; Zazimalova, E.; Santrucek, J. Light-induced STOMAGEN-mediated stomatal development in *Arabidopsis* leaves. *J. Exp. Bot.* **2015**, *66*, 4621–4630. [[CrossRef](#)] [[PubMed](#)]
60. Aasamaa, K.; Aphalo, P.J. The acclimation of *Tilia cordata* stomatal opening in response to light, and stomatal anatomy to vegetational shade and its components. *Tree Physiol.* **2017**, *37*, 209–219. [[CrossRef](#)] [[PubMed](#)]
61. Bertolino, L.T.; Caine, R.S.; Gray, J.E. Impact of stomatal density and morphology on water-use efficiency in a changing world. *Front. Plant Sci.* **2019**, *10*, 225. [[CrossRef](#)] [[PubMed](#)]
62. Kardiman, R.; Raebild, A. Relationship between stomatal density, size and speed of opening in Sumatran rainforest species. *Tree Physiol.* **2018**, *38*, 696–705. [[CrossRef](#)] [[PubMed](#)]
63. Elango, T.; Jeyaraj, A.; Dayalan, H.; Arul, S.; Govindasamy, R.; Prathap, K.; Li, X. Influence of shading intensity on chlorophyll, carotenoid and metabolites biosynthesis to improve the quality of green tea: A review. *Energy Nexus* **2023**, *12*, 100241. [[CrossRef](#)]
64. Ma, Z.; Li, S.; Zhang, M.; Jiang, S.; Xiao, Y. Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of *Anoectochilus* plants. *HortScience* **2010**, *45*, 863–867. [[CrossRef](#)]
65. Xue, G.; Wu, J.; Zhou, B.; Zhu, X.; Zeng, J.; Ma, Y.; Wang, Y.; Jia, H. Effects of shading on the growth and photosynthetic fluorescence characteristics of *Castanopsis hystrix* seedlings of top community-building species in southern subtropical China. *Forests* **2023**, *14*, 1659. [[CrossRef](#)]
66. Kume, A.; Akitsu, T.; Nasahara, K.N. Why is chlorophyll b only used in light-harvesting systems? *J. Plant Res.* **2018**, *131*, 961–972. [[CrossRef](#)] [[PubMed](#)]
67. Khan, I.; Zada, A.; Jia, T.; Hu, X. Effect of the enhanced production of chlorophyll b on the light acclimation of tomato. *Int. J. Mol. Sci.* **2023**, *24*, 3377. [[CrossRef](#)] [[PubMed](#)]
68. Dziwulska-Hunek, A.; Mysliwa-Kurdziel, B.; Matwijczuk, A.; Szymanek, M. A case study in photosynthetic parameters of perennial plants growing in natural conditions. *BMC Plant Biol.* **2025**, *25*, 1044. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.